(4) W. Hartmann and R. Steinmetz, *Chem. Ber.*, **100**, 217 (1967); W. Hartmann, *ibid.*, **101**, 1643 (1968); W. Hartmann, H.-M. Fischler, and H.-G. Heine, *Tetrahedron Lett.*, 853 (1972); S. Farid, D. Hess, and C. H. Kruch, *Chem. Ber.*, **100**, 3266 (1967); D. Helmlinger, P. de Mayo, M. Nye, L. Westfelt, and R. B. Yeats, *Tetrahedron Lett.*, 349 (1970); R. H. Grubbs, *J. Amer. Chem. Soc.*, **92**, 6693 (1970); J. Tancrede and M. Rosenblum, *Synthesis*, 219 (1971).
(5) J. Daub and V. Trautz, *Tetrahedron Lett.*, 3265 (1970); R. Criegee and P. Becher, *Chem. Ber.*, **90**, 2516 (1957); H. D. Scharf, W. Küsters, and J. Flischhauser, *ibid.*, **104**, 3016 (1971); *Angew. Chem.*, **83**, 335 (1971); M. Z. Haq, *J. Org. Chem.*, **37**, 3015 (1972); C. R. Kowarski and S. Sarel, *ibid.*, **38**, 117 (1973). For recent papers on substituted vinylene carbonates, see P. Lechtken and G. Hesse, *Justus Liebigs Ann. Chem.*, **754**, 1 (1972); H.-D. Scharf and R. Klar, *Chem. Ber.*, **105**, 564, 575 (1972); K.-R. Stahlke, H.-G. Heine, and W. Hartmann, *Justus Liebigs Ann. Chem.*, **764**, 116 (1972).
(6) M. S. Newman and R. W. Addor, *J. Amer. Chem. Soc.*, **75**, 1263 (1953); **77**, 3789 (1955).
(7) C. Walling, "Free Radicals in Solution," Wiley, New York, N. Y., 1966, p 201; W. A. Pryor, "Free Radicals," McGraw-Hill, New York, N. Y., 1966, p 201; B. A. Bohm and P. I. Abell, *Chem. Rev.*, **62**, 599 (1962).
(8) Chromatography on alumina caused rapid hydrolysis of carbonic ester groups to alcohols whose purification was quite difficult.
(9) C. Walling, "Free Radicals in Solution," Wiley, New York, N. Y., 1957, p 97.
(10) M. Rogozinski, L. M. Shorr, U. Hashman, and D. A. Barlas, *J. Org. Chem.*, **33**, 3859 (1968).
(11) J. G. Traynham, A. G. Lane, and N. S. Bhacca, *J. Org. Chem.*, **34**, 1302 (1969); L. H. Gale, *ibid.*, **34**, 81 (1969).
(12) Though no general pattern can be discerned regarding the relative magnitudes of cis and trans vicinal coupling constants in five-membered rings, the data, in some instances,[13,14] may prove of use for empirical assignment of configuration. *Cf.* L. M. Jackman and S. Sternhell, "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," 2nd ed, Pergamon Press, Elmsford, N. Y., 1969, p 280.
(13) T. D. Inch and N. Williams, *J. Chem. Soc. C*, 262 (1970); G. E. Wilson, Jr., M. G. Huang, and F. A. Bovey, *J. Amer. Chem. Soc.*, **92**, 5907 (1970).
(14) F. A. L. Anet, *J. Amer. Chem. Soc.*, **84**, 747 (1962).
(15) L. E. Erickson, *ibid.*, **87**, 1867 (1965). Additional data are provided by trans and cis isomers of 4-cyano-5-trichloromethyl-1,3-dioxolan-2-one, which show J_{vic} = 4.0 and 8.0 Hz, respectively (H. Takahata of this laboratory).
(16) T. Kunieda, T. Tamura, and T. Takizawa, *Chem. Commun.*, 885 (1972).
(17) J. S. Brimacombe, F. Hundey, and L. C. N. Tucker, *J. Chem. Soc. C*, 1381 (1968).
(18) P. A. Levene and A. L. Raymond, *J. Biol. Chem.*, **102**, 317 (1933); R. S. Tipson, H. S. Isbell, and J. E. Stewart, *J. Res. Nat. Bur. Stand., Sect. A*, **62**, 267 (1959).
(19) W. W. Hartman and E. E. Dreger, "Organic Syntheses," Collect. Vol. I, Wiley, New York, N. Y., 1956, p 357.
(20) Paper chromatography was performed by the ascending technique on Toyo No. 51 paper using *n*-BuOH–pyridine–H_2O (6:4:3) as a developing solvent and the spot was developed with the diphenylamine–aniline reagent. *Cf.* R. W. Bailey and E. J. Bourne, *J. Chromatogr.*, **4**, 206 (1960); J. L. Buchan and R. I. Savage, *Analyst*, **77**, 401 (1952).
(21) Trimethylsilates were obtained by the usual procedure using a mixture of trimethylsilyl chloride and hexamethyldisilazane. *Cf.* C. C. Sweeley, R. Bentley, M. Makita, and W. W. Wells, *J. Amer. Chem. Soc.*, **85**, 2497 (1963). Gas chromatography was performed with column B at 140° using helium as carrier gas.

Preparation and Use of Benzhydrylamine Polymers in Peptide Synthesis. II. Syntheses of Thyrotropin Releasing Hormone, Thyrocalcitonin 26–32, and Eledoisin[1]

Pietro G. Pietta,[2] P. F. Cavallo, Kiyohisa Takahashi,[3] and Garland R. Marshall*[4]

Instituto di Chimica Organica dell'Universita, Milano, Italy, and the Departments of Medicine and of Physiology and Biophysics, Washington University School of Medicine, St. Louis, Missouri 63110

Received June 25, 1973

Three procedures have been developed for the synthesis of a benzhydrylamine polymer for the preparation of C-terminal amide peptides by solid-phase synthesis. From a common keto intermediate, prepared by acylation of polysterene–1% divinylbenzene with benzoyl chloride, the desired product can be obtained directly by the Leukart reaction, by reduction of an oxime intermediate, or by ammonolysis of the benzhydryl bromide intermediate. The application of this support to the syntheses of the wide range of peptide hormones possessing a C-terminal amide is illustrated by the syntheses of TRH, the C-terminal heptapeptide fragment of thyrocalcitonin, and the endecapeptide, eledoisin.

The original synthesis of a biologically active peptide hormone was that of oxytocin by du Vigneaud.[5] Both oxytocin and antidiuretic hormone are of neurohypophyseal origin and are characterized by the presence of the C-terminal amide group. Recent advances in endocrinology have led to the isolation and sequence analysis of a number of other peptides which are also characterized by the presence of the C-terminal amide group. The presence of the masked carboxyl function may serve to protect these peptides from degradation by exopeptidases with carboxypeptidase specificity. Examples of such physiologically important peptides would be thyrotropin releasing hormone (TRH) and follicle stimulating hormone-luteinizing hormone-releasing hormone (FSH-LH-RH) from the hypothalamus,[6] gastrin,[7] cholecystokinin–pancreozymin and secretin from the gastrointestinal tract,[8] thyrocalcitonin from the thyroid,[9] and substance **P**, which was also isolated from the hypothalamus.[10]

Concurrent advances in peptide synthesis have seen the development of the solid-phase method.[11] As originally outlined by Merrifield[12] and most commonly employed, the solid-phase method utilizes a polymeric benzyl ester for carboxyl protection. Cleavage of this link to the polymeric support by ammonolysis either with NH_3–CH_3OH or NH_3–dimethylformamide[13-15] or with liquid ammonia[16] to give the desired peptide amide has been reported. Minor difficulties with the side reaction of transesterification or with hindered release of product have been noted.[17-20] A more serious consideration is the problem of protection of side chain carboxyl groups such as those of glutamic or aspartic acid residues. Restriction of side chain protection to the labile *tert*-butyl esters which are resistant to ammonolysis imposes concomitant restraints on the choice of amino group protection to ones even more labile.

In order to circumvent these problems when the desired product possesses a C-terminal amide function, we have conceived of a new type of polymeric carboxyl protecting group.[21] In this case, the covalent link to the support is through an amide bond between the C-terminal amino acid residue and the amine function of the polymer. Selective cleavage of the bond between the amino group and the support allows the peptide to be cleaved with the C-terminal amide function intact. The benzhydrylamine (diphenylmethylamine) support is the first of such supports which allow the selective placement of the amide function. Three methods[22] for the preparation of this sup-

port are compared and the application of this approach is demonstrated by the syntheses of the C-terminal heptapeptide fragment of thyrocalcitonin and of two biologically active peptides, TRH and eledoisin,[23] a hypotensive peptide isolated[24] from the salivary glands of the mollusk *Eledone* and similar in structure to substance **P**.

Results and Discussion

Three synthetic routes to the benzhydrylamine support are outlined in Figure 1. A common intermediate is the phenyl keto derivative of polystyrene-1%-divinylbenzene copolymer prepared by Friedel–Craft acylation of the polymer with benzoyl chloride. Initial preparation was by reduction of the ketone with sodium borohydride or sodium bis(2-methoxyethoxy)aluminum hydride;[25] treatment of the resulting carbinol with HBr in methylene chloride gave the benzhydryl bromide polymer essentially as outlined by Southard and coworkers.[26] The desired amine derivative was obtained by treatment with ammonia in methylene chloride. The amine content was determined by titration as described by Dorman,[27] by substitution with an amino acid and subsequent hydrolysis and amino acid analysis, or by the procedure of Esko.[28]

A second procedure involved the preparation of an intermediate oxime resin by treatment of the ketone polymer with hydroxylamine hydrochloride in ethanol–pyridine. This intermediate was easily converted to the desired benzhydrylamine support by reduction with sodium bis(2-methoxyethoxy)aluminum hydride in benzene or with lithium aluminum hydride in ether.

The third procedure[29] is direct reductive amination by the Leukart reaction of the ketone polymer with ammonium formate and hydrolysis of the formyl derivative to give the free amine. While this procedure is the most direct, it also has been the most sensitive to reaction conditions. For this reason, the preparation of the intermediate oxime has been favored in our laboratories except when the benzhydryl bromide polymer, which is an intermediate in the first scheme, is also desired.

For any modification of the solid-phase procedure to be useful and to gain acceptance, it should offer advantages over existing techniques and be applicable to the wide range of biologically active peptides. The amide support offers decided advantages over the benzyl ester support in the synthesis of C-terminal amide peptides. It allows the selective placement of the amide function at the C-terminal residue in the presence of a side chain carboxyl group as has been demonstrated in the synthesis of gastrin tetrapeptide.[22] The amide bond to the polymer offers the advantage of being synthesized under identical conditions with those of the subsequent peptide bonds. In other words, a particular amino acid polymer does not have to be prepared under conditions different, *i.e.*, refluxing ethanol, from those used for the subsequent peptide synthesis. The presence of excess chloromethyl groups and the formation of quaternary amine is also eliminated. Another advantage is the elimination of possible side chain alkylation by the chloromethyl group of such amino acids as methionine and histidine, which has presented difficulties in polymer attachment procedures. The stability of the amide linkage compared to the ester bond also offers advantages. Loss of the peptide from the support by ester hydrolysis during synthesis and by intramolecular aminolysis[30] to give the diketopiperazine when the C-terminal amino acid is an *N*-methyl derivative such as proline should be reduced. In addition, removal of the peptide from the support is by hydrogen fluoride treatment, which removes a wide variety of protecting groups and, in most cases, would simplify the synthetic procedure.

The syntheses of TRH[31] and thyrocalcitonin 26–32 demonstrate the usefulness of this approach with C-terminal proline peptides. An overall yield of 36% of TRH based on the original amount of amine on the polymer and a yield on cleavage of 88% of the proline originally bound in the synthesis of the calcitonin heptapeptide indicate that very little, if any, formation of proline diketopiperazine with resulting cleavage from the support had occurred. In the case of TRH, comparison of the synthetic product prepared on the polymer with authentic TRH prepared by classical solution procedures[32] gave identical results on thin layer chromatography in five different solvent systems and the biological activity (Table I) of the two compounds is similar. The successful synthesis of the C-terminal heptapeptide of thyrocalcitonin demonstrates the applicability of solid phase, in general, and this approach, in particular, to that hormone.

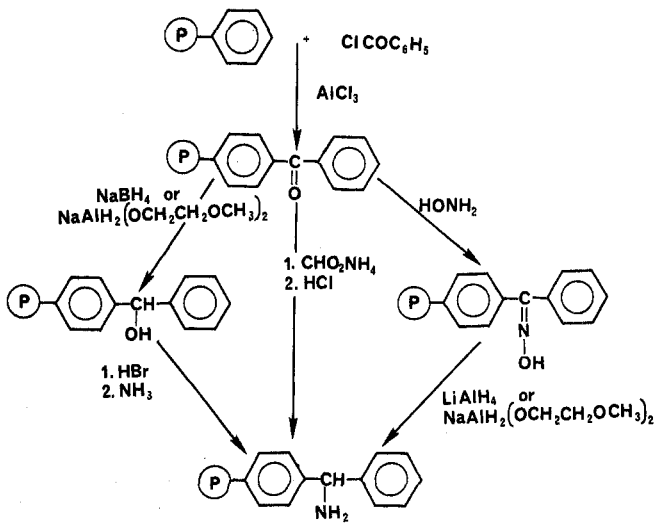

Figure 1. Synthesis of benzhydrylamine polymer by three different procedures.

Table I
Comparison of the Present Synthetic TRH to Standard TRH

	Ng/mouse	CPM
Saline		−24 ± 42
Synthetic TRH	5.0	214 ± 66
	25.0	860 ± 264
Standard TRH	5.0	300 ± 72
	25.0	930 ± 286

The synthesis of the endecapeptide, eledoisin,[33] demonstrates the applicability of this approach to peptides in the eledoisin, physaelamin, and substance **P**[34] series. The problems of S-alkylation associated with attachment of C-terminal methionine to the normal chloromethyl support are eliminated with the use of the benzhydrylamine support. The chemical properties and biological activity of this peptide were also compared with those of an authentic standard and found to be similar. The synthesis of other C-terminal methionine amide peptides isolated from amphibian skin such as alytesin, bombesin,[35] and ranatensin[36] is also feasible using the benzhydrylamine support. In addition, two toxic peptides, apamin[37] and melittin,[38] have been isolated from bee venom which contain a C-terminal histidine amide and glutamine amide, respectively. The synthesis of analogs of these compounds should also be facilitated by the use of the benzhydrylamine support.

The question of increased stability of the growing peptide chain to deprotection has been examined.[22] These successful syntheses are certainly consistent with this hypothesis, but do little to substantiate it. The use of the benzhydrylamine polymer in the synthesis of longer pep-

tides should confirm this suggestion. In this connection, it is not necessary to require a C-terminal amide function for the use of this support. The use of this support as polymeric side chain protection[11] by the attachment of either the β-carboxyl of aspartic acid to give an asparagine derivative or the γ-carboxyl of glutamic acid to give a glutamine derivative would allow elongation at either the carboxyl or amino end. This concept has been amply illustrated,[39] but not yet utilized for the synthesis of longer peptide chains.

Experimental Section

All solvents used were purified according to standard procedures.[40] Acid hydrolyses of peptide polymer were performed according to Scotchler, *et al.*[41] Acid hydrolysates of free peptides were prepared using 6 *N* HCl (110°, 16 hr) and the amino acid composition was determined with an Auto-Analyzer or a Spinco 120C. Optical rotations were determined with a Perkin-Elmer polarimeter, Model 141, or a Cary 60. Melting points (uncorrected) were determined in capillary tubes in a Tottoli melting point apparatus (manufactured by Buchi). Thin layer chromatography (tlc) was done on silica gel G plates (Analtech) in the following systems: (I) methanol–chloroform (60:30); (II) chloroform–methanol–concentrated ammonia (60:45:20); (III) 1-butanol–ethyl acetate–acetic acid–water (1:1:1:1); (IV) chloroform–methanol–38% acetic acid (60:40:20); (V) 1-butanol–acetic acid–water (4:1:1). The bioassay method for TRH was that described by Bowers and Shally.[42]

Benzoyl-Polystyrene-1% Divinylbenzene (Keto Polymer). Cross-linked polystyrene (1%, 30 g, BioBead SX1) was suspended in 200 ml of nitrobenzene containing 10 ml (86 mmol) of benzoyl chloride. Anhydrous aluminum chloride (16 g, 160 mmol) was added slowly with vigorous stirring. The reaction mixture was then refluxed at 90–100° for 24 hr while stirring. The polymer was filtered and washed as follows: nitrobenzene (3 × 100 ml), ethanol (3 × 100 ml), ethanol–water (1:1) (3 × 100 ml), ethanol (3 × 100 ml), CH_2Cl_2 (3 × 100 ml). After drying *in vacuo*, a strong carbonyl absorption at 1670 cm^{-1} was observed by ir.

Phenylketoximyl-Polystyrene-1% Divinylbenzene (Oxime Polymer). Keto polymer (4 g) was suspended in 40 ml of ethanol and 5 ml of pyridine. Hydroxylamine hydrochloride (5 g, 73 mmol) was added and the mixture was refluxed with stirring for 24 hr. The polymer was filtered and washed as follows: ethanol (3 × 50 ml), ethanol–water (1:1) (3 × 50 ml), ethanol (3 × 50 ml), CH_2Cl_2 (3 × 50 ml). After drying *in vacuo* at 45°, ir (KBr pellet) showed absence of the carbonyl absorption at 1670 cm^{-1}.

Phenylhydroxymethyl-Polystyrene-1% Divinylbenzene (Hydroxy Polymer). To a suspension of keto polymer (12 g) in 100 ml of 2-(2-ethoxy)ethanol was added 3 g (79 mmol) of $NaBH_4$ portionwise while stirring at 55°. After 4 hr, the mixture was filtered and washed as described above (see keto polymer) with the substitution of 2-(2-ethoxyethoxy)ethanol for nitrobenzene. Ir showed the disappearance of the absorption at 1670 cm^{-1} and the appearance of a strong band at 3500 cm^{-1} (OH).

Phenylbromomethyl-Polystyrene-1% Divinylbenzene (Benzhydryl Bromide Polymer). A suspension of hydroxy polymer (11 g) in 250 ml of methylene chloride was cooled to 0° and HBr gas was bubbled into the mixture for 4 hr. The polymer was allowed to warm to room temperature, filtered, and washed with methylene chloride (3 × 40 ml) and ethanol (3 × 40 ml). An aliquot of the polymer was heated in pyridine for 2 hr and the bromine content was determined by Volhard titration to be 0.6 mmol/g.

Phenylaminomethyl-Polystyrene-1% Divinylbenzene (Benzhydrylamine Polymer). A. From the Benzhydryl Bromide Polymer. Phenylbromomethyl polymer (10 g) was suspended in 250 ml of methylene chloride cooled to 0°. Ammonia was bubbled through this mixture for 8 hr. The reaction mixture was stirred for an additional 12 hr at 0°. The polymer was then filtered, washed, and dried as indicated for the bromo polymer and contained 0.6 mmol of NH_2/g as the hydrobromide salt.

B. From the Oxime Polymer. 1. $LiAlH_4$ Reduction. Oxime polymer (4 g) was suspended in 50 ml of ether and added to a slurry of $LiAlH_4$ (3.79 g) in 300 ml of anhydrous ether. The reaction mixture was refluxed for 24 hr. Excess $LiAlH_4$ was treated with ethanol, then 10% concentrated HCl was added to dissolve the reduction by-product. The polymer was filtered, and washed repeatedly with 10% HCl, ethanol (3 × 50 ml), and CH_2Cl_2 (3 × 50 ml). The polymer was washed with 10% triethylamine in $CHCl_3$ to convert the hydrochloride to the free amine. After drying, the ir indicated broad absorption at 3500–3100 cm^{-1} (amine). A test sample was substituted with Boc-Tyr(Bzl) at 0.52 mmol/g.

2. $NaAlH_2(OCH_2CH_2OCH_3)_2$ Reduction. Oxime polymer (3 g, solid) was added to a stirred solution of $NaAlH_2(OCH_2CH_2OCH_3)_2$ (12 mmol) in 50 ml of benzene at 60°. The mixture was allowed to react overnight, when excess reagent was treated with dilute HCl. The polymer was then filtered, and washed with benzene (3 × 50 ml), ethanol (3 × 50 ml), H_2O (3 × 50 ml), ethanol (3 × 50 ml), and CH_2Cl_2 (3 × 50 ml). The polymer was washed with 10% triethylamine in $CHCl_3$ to convert the hydrochloride to the free amine. After drying, the ir and test substitution gave identical results with those prepared above.

C. By Leukart Reduction. Keto polymer (2.0 g) was suspended in 20 ml of nitrobenzene. To a three-necked flask fitted with a thermometer and reflux condenser containing 8.0 ml (0.2 mol) of 97% formic acid was added 13.5 ml (0.2 mol) of 28% NH_4OH. The contents were stirred and heated to distil off water until the temperature of the mixture reached 160°. Then the suspension of keto polymer in nitrobenzene was added. The reaction mixture was stirred for 22 hr at 160–170°. Then 20 ml of concentrated HCl was added to the flask and the mixture was refluxed for 8 hr. The mixture was allowed to stand at room temperature for an additional 10 hr; 50 ml of H_2O was added; and the polymer product was filtered and washed with nitrobenzene (3 × 20 ml), ethanol (3 × 20 ml), ethanol–H_2O (1:1) (3 × 20 ml), ethanol (3 × 20 ml), and CH_2Cl_2 (3 × 20 ml). The polymer was dried *in vacuo* at 45° to give 2.23 g of benzhydrylamine polymer as the hydrochloride. Treatment with 10% triethylamine in $CHCl_3$ gave the free amine. Ir indicated loss of the CO absorption (1720 cm^{-1}) and presence of the NH_2 band. A sample was treated with acetic anhydride and Et_3N and ir showed reappearance of CO absorption. To another sample, Boc-glycine was added by the normal procedure and substitution of 0.41 mmol/g was obtained.

L-Pyroglutamyl-L-histidyl-L-proline Amide (TRH). Benzhydrylamine polymer (2 g, 0.47 mmol of amine/g of resin) prepared by the Leukart method was loaded into the reaction vessel, swollen in CH_2Cl_2 for a few minutes, and washed with CH_2Cl_2 (3 × 20 ml). Further steps of peptide synthesis were as follows. (1) Boc-L-Pro (2.35 mmol) dissolved in CH_2Cl_2 was added to the reaction vessel and mixed with resin for a few minutes. Then an equimolar amount of *N,N*-dicyclohexylcarbodiimide (DCCI) in CH_2Cl_2 was added and the reaction mixture was maintained for 2 hr. (2) Resin was washed with CH_2Cl_2 (3 × 20 ml) and then the Boc protecting group was removed by 20 ml of 50% (v/v) trifluoroacetic acid in CH_2Cl_2 for 1 hr. Deprotection was followed by three washings each (20 ml) with CH_2Cl_2, 95% (v/v) *tert*-butyl alcohol in CH_2Cl_2, and CH_2Cl_2. (3) Resin was washed with $CHCl_3$ (3 × 20 ml) and then was neutralized by 10% (v/v) triethylamine in $CHCl_3$ for 10 min followed by washes with $CHCl_3$ and CH_2Cl_2 (3 × 20 ml). (4) Boc-Im-DNP-L-His (2.35 mmol) was attached to the resin as for Boc-Pro and deprotection and neutralization were carried out the same as in 2 and 3. (5) Neutralization after the second addition was followed by washes (3 × 20 ml) with CH_2Cl_2 and DMF. L-Pyroglutamic acid and DCCI (2.35 mmol) dissolved in DMF were added to the reaction vessel and reaction was maintained for 2 hr. The resin was then washed with DMF (3 × 20 ml). (6) To remove DNP from the histidine imidazole ring, thiophenol (20 molar excess) in DMF was added to the reaction vessel and shaken for 1 hr.[43] The resin was washed with DMF and CH_2Cl_2 three times, respectively. (7) The final product was cleaved from the resin in an apparatus described by Pourchot and Johnson[44] with HF as described below. After the final wash of resin with CH_2Cl_2, it was vacuum dried overnight and then transferred into a polypropylene reaction vessel for HF cleavage. Anisole (3 ml) was added to protect the product. After the reaction and reservoir vessels were placed on the HF line, nitrogen gas was passed through the HF line. HF was distilled into the reservoir vessel (20 ml) and then transferred to the reaction vessel. The reaction mixture was stirred for 1 hr at room temperature. After the reaction was completed, the HF was evaporated from the reaction vessel with a stream of nitrogen gas. The cleavage mixture was dried completely *in vacuo*. The crude product was then extracted with 1% acetic acid and freeze-dried immediately. (8) Purification of the peptide was by means of countercurrent distribution (CCD) in 1-butanol–acetic acid–water (4:1:5) with 200 transfers. After completion of the CCD run, the peptide peaks were located by the Pauly reaction. The peak fraction (K = 0.10) was pooled, the 1-butanol was evaporated *in vacuo*, and the remaining liquid was lyophilized; 121 mg (36% yield) of white powder was obtained. The purified compound was homogenous on tlc in five different systems: R_f I 0.52, R_f II 0.69, R_f III 0.54, R_f IV 0.56, R_f V 0.21. Standard synthetic TRH supplied by Abbott Labs showed identical behavior when compared with the product

on tlc in the above systems. Amino acid ratios were Glu, 1.0; His, 1.1; Pro, 1.0.

Anal. Calcd for $C_{16}H_{22}O_4N_6 \cdot CH_3CO_2H \cdot 1\frac{1}{2}H_2O$: C, 48.40; H, 6.5; N, 18.7. Found: C, 48.16; H, 6.13; N, 18.64.

Boc-L-prolylbenzhydrylamine Polymer. A solution of 0.9 g (4.2 mmol) of Boc-L-proline in 5 ml of methylene chloride was added to 2 g of benzhydrylamine polymer (prepared from oxime polymer) suspended in 5 ml of methylene chloride. After 5 min of shaking, 0.86 g (4.2 mmol) of DCCI in 2 ml of methylene chloride was added and the mixture was shaken for 2 hr. The solvent was filtered off and the resin was washed with methylene chloride (three times). This coupling was repeated using the same amount of Boc-L-proline and of DCCI. The resin was then treated with acetic anhydride to mask the remaining amino groups. After washing with methylene chloride (three times), ethanol (three times), and again with methylene chloride (three times), the resin was dried *in vacuo*, yield 2.14 g. Amino acid analysis of an acid hydrolysate showed the product to contain 0.29 mmol of proline/g coupled to the resin.

Boc-glycyl-L-phenylalanylglycyl-L-prolyl-γ-benzyl-L-glutamyl-O-(2,2,2-trifluoro-1-benzyloxycarbonylaminoethyl)-L-threonyl-L-prolylbenzhydrylamine Polymer. The Boc-L-prolylbenzhydrylamine polymer (1.2 g) was placed in the reaction vessel. The following cycle of deprotection, neutralization, and coupling was carried for the introduction of each new residue: (1) three washings with 10-ml portions of glacial acetic acid; (2) cleavage of the Boc group by treatment with 1 *N* HCl in glacial acetic acid (10 ml) for 5 min followed by 10 ml for 30 min; (3) three washings with 10-ml portions of glacial acetic acid; (4) three washings with 10-ml portions of absolute ethanol; (5) three washings with 10-ml portions of methylene chloride; (6) neutralization of the hydrochloride with 1 ml of triethylamine in 9 ml of methylene chloride for 10 min; (7) three washings with 10-ml portions of methylene chloride; (8) addition of 1.04 mmol (300% excess) of the appropriate Boc-amino acid in 8 ml of methylene chloride and mixing for 5 min; (9) addition of 1.04 mmol of DCCI in 2 ml of methylene chloride, followed by a reaction period of 3 hr; (10) three washings with 10-ml portions of methylene chloride; (11) three washings with 10-ml portions of absolute ethanol.

The polypeptide resin was washed three times with 10-ml portions of methylene chloride followed by three washings with 10-ml portions of absolute ethanol and dried *in vacuo*, yield 1.57 g (0.27 mmol of peptide/g).

Glycyl-L-phenylalanylglycyl-L-prolyl-L-glutamyl-L-threonyl-L-prolinamide (Thyrocalcitonin 26–32). The protected peptide resin (1.57 g) was treated with hydrogen fluoride (10 ml) and excess anisole (1 ml) and stirred at 0° for 2 hr. The reaction mixture was then brought to room temperature and the hydrogen fluoride was allowed to evaporate. The crude peptide was separated from the resin by repeated washing of the resin with trifluoroacetic acid. After evaporation of the combined washings under reduced pressure, the residue was treated with dry ether and filtered. The precipitate was washed three times with ether and dried *in vacuo*. The yield of the crude heptapeptide amide from the cleavage was 214 mg (88% on the amount of proline originally bound to the resin).

The crude heptapeptide amide was dissolved in 15 ml of 1 *M* acetic acid and applied to a Sephadex LH-20 column (4.5 × 40 cm) which had been equilibrated with 1 *M* acetic acid. The column was eluted with the same solvent and 90 fractions of 7.5 ml each were collected. The maximum of the desired peptide was in tube 44, as shown by measurement of ninhydrin color values of the various fractions. The fractions corresponding to this maximum were pooled and lyophilized to give 99 mg of white powder.

This lyophilized material was subjected again to gel filtration under the same conditions. The shape of the distribution curve suggested that the peptide was homogeneous. The fractions corresponding to the maximum were combined and lyophilized, yield 89 mg (36% overall yield).

In paper chromatography (Whatman No. 4) in the solvent system 1-butanol–acetic acid–water (4:1:1), the peptide showed a single spot (R_f 0.32) visible with ninhydrin. In paper electrophoresis at pH 1.9 in the solvent system formic acid–acetic acid–water (15:10:75), it moved as a spot toward the cathode with migration relative to histidine of 0.23 and relative to leucine of 0.37, $[\alpha]^{20}D$ −0.75° (*c* 1, acetic acid). Amino acid analysis gave Gly, 1.97; Phe, 1.00; Pro, 2.02; Glu, 1.00; Thr, 0.93.

Boc-L-methionylbenzhydrylamine Polymer. Benzhydrylamine polymer (2 g) was treated with Boc-L-methionine (1.04 g, 4.2 mmol) and DCCI (0.86 g, 4.2 mmol) as described for Boc-L-prolyl benzhydrylamine polymer. Amino acid analysis of an acid hydrolysate showed the product to contain 0.26 mmol of methionine/g of coupled resin.

Boc-L-pyroglutamyl-L-prolyl-O-(2,2,2-trifluoro-1-benzyloxycarbonylaminoethyl)-L-seryl-N^{ϵ}-benzyloxycarbonyl-L-lysyl-β-benzyl-L-aspartyl-L-alanyl-L-phenylalanyl-L-isoleucylglycyl-L-leucyl-L-methionylbenzhydrylamine Polymer. The Boc-L-methionylbenzhydrylamine polymer (1.5 g) was placed in the reaction vessel. The same cycle described for the the synthesis of C-terminal heptapeptide of thyrocalcitonin was carried out for the introduction of each new residue.

The fully protected endecapeptide resin weight was 2.12 g (90% based on the amount of methionine originally bound to the resin).

L-Pyroglutamyl-L-prolyl-L-seryl-L-lysyl-L-aspartyl-L-alanyl-L-phenylalanyl-L-isoleucylglycyl-L-leucyl-L-methionine Amide (Eledoisin). Treatment of protected peptide resin (2.12 g) with hydrogen fluoride and extraction of the reaction mixture with trifluoroacetic acid as previously described gave 415 mg of crude peptide.

Crude peptide (200 mg) was purified by passing through a Sephadex LH-20 column (3 × 100 cm) which had been equilibrated with 1 *M* acetic acid. The column was eluted with the same solvent and 120 fractions of 6 ml each were collected. Eledoisin appeared to be in fractions 44–52, which were pooled and lyophilized. A further purification under the same conditions gave 55 mg of homogeneous product (18.5% yield), mp 230° dec, $[\alpha]^{22}D$ −48° (*c* 2, glacial acetic acid).

In paper chromatography (Whatman No. 4) in the solvent system 1-butanol–acetic acid–water (4:1:5), the peptide showed a single spot (R_f 0.4). In paper electrophoresis at pH 1.9 in the solvent system formic acid–acetic acid–water (15:10:75), it moved as a spot toward the cathode with migration relative to histidine of 0.34 and relative to leucine of 0.50.

Amino acid analysis gave Lys, 0.88; Asp, 0.93; Ser, 0.97; Glu, 0.98; Pro, 1.04; Gly, 1.00; Ala, 1.06; Met, 0.99; Ileu, 1.01; Leu, 1.08; Phe, 1.03.

The synthetic product showed the same activity as natural eledoisin when tested in rabbit blood pressure and guinea pig ileum assays.

Registry No. TRH, 24305-27-9; thyrocalcitonin 26–32, 42790-39-6; eledoisin, 69-25-0.

References and Notes

(1) This work was supported by Grant AM 13025 from the USPHS and the Consiglio Nazionale delle Ricerche of Italy.
(2) Recipient of a NATO Fellowship during which this work was initiated.
(3) Trainee in Metabolism (Grant AMO-1921).
(4) Established Investigator, American Heart Association.
(5) V. du Vigneaud, C. Ressler, J. M. Swan, C. W. Roberts, P. G. Katsoyannis, and S. Gordon, *J. Amer. Chem. Soc.*, **75**, 4879 (1953).
(6) (a) R. Guillemin in "Chemistry and Biology of Peptides," J. Meienhofer, Ed., Ann Arbor Science Publications, 1972, p 585; (b) A. V. Schally, A. Arimura, and A. J. Kastin, *Science*, **179**, 341 (1973).
(7) H. Gregory, P. M. Hardy, D. S. Jones, G. W. Kenner, and R. C. Sheppard, *Nature (London)*, **204**, 931 (1964).
(8) J. E. Jorpes, *Gastroenterology*, **55**, 157 (1968).
(9) J. T. Potts, Jr., H. D. Niall, H. T. Keutmann, H. B. Brewer, Jr., and L. J. Deftos, *Proc. Nat. Acad. Sci. U. S.*, **59**, 1321 (1968).
(10) M. M. Chang, S. E. Leeman, and H. D. Niall, *Nature New Biol.*, **232**, 86 (1971).
(11) G. R. Marshall and R. B. Merrifield in "Biochemical Aspects of Reactions on Solid Supports," G. Stark, Ed., Academic Press, New York, N. Y., 1971.
(12) R. B. Merrifield, *J. Amer. Chem. Soc.*, **85**, 2149 (1963).
(13) (a) H. Takashima, V. du Vigneaud, and R. B. Merrifield, *J. Amer. Chem. Soc.*, **90**, 1323 (1968); (b) M. Manning, *ibid.*, **90**, 1348 (1968).
(14) H. C. Beyerman, P. Kranenburg, and J. L. M. Syrier, *Recl. Trav. Chim. Pays-Bas*, **90**, 791 (1971).
(15) E. Bayer, E. Breitmaier, G. Jung, and W. Paar, *Hoppe-Syeler's Z. Physiol. Chem.*, **352**, 759 (1972).
(16) W. Parr, C. Yang, and G. Holzer, *Tetrahedron Lett.*, 101 (1972).
(17) M. Bodanszky and J. T. Sheehan, *Chem. Ind. (London)*, 1597 (1966).
(18) W. Kessler and B. Iselin, *Helv. Chim. Acta*, **49**, 1330 (1966).
(19) H. C. Beyerman, H. Hindriks, and E. W. B. DeLeer, *Chem. Commun.*, 1668 (1968).
(20) S. Visser, J. Roeloffs, K. E. T. Kerlinga, and E. Havinga, *Recl. Trav. Chim. Pays-Bas*, **87**, 559 (1968).
(21) This was the subject of a preliminary communication by P. G. Pietta and G. R. Marshall, *Chem. Commun.*, 650 (1970). The support is based on the amide protecting group, diphenylmethyl (benzhydryl), used in solution work by S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Jap.*, **40**, 2164 (1967), and by S. Ariely, M. Fridkin, and A. Patchornik, *Biopolymers*, **7**, 417 (1969).

(22) The first paper of this series outlines the preparations of the polymer and its application to the synthesis of gastrin tetrapeptide. P. G. Pietta, P. F. Cavallo, G. R. Marshall, and M. Pace, *Gazz. Chim. Ital.*, in press.
(23) This work was the subject of a preliminary communication at the 11th European Peptide Symposium, Vienna, 1971. See P. G. Pietta, P. F. Cavallo, and G. R. Marshall, "Peptides 1971," H. Nesvadba, Ed., North-Holland Publishing Co., Amsterdam, 1972.
(24) V. Erspamer and A. Anastasi, *Experientia*, **18**, 58 (1962).
(25) M. Capka, V. Chvalovsky, K. Kochloefl, and M. Kraus, *Collect. Czech. Chem. Commun.*, **34**, 118 (1969).
(26) G. L. Southard, G. S. Brooke, and J. M. Pettee, *Tetrahedron Lett.*, 3505 (1969).
(27) L. C. Dorman, *Tetrahedron Lett.*, 2319 (1969).
(28) K. Esko, S. Karlsson, and J. Porath, *Acta Chem. Scand.*, **22**, 3342 (1968).
(29) This procedure has also been used successfully in the preparation of benhydrylamine polymer for the synthesis of FSH-LH-RH: P. Rivaille, A. Robinson, M. Kamen, and G. Milhaud, *Helv. Chim. Acta*, **54**, 2772 (1971).
(30) (a) B. F. Gisin and R. B. Merrifield, *J. Amer. Chem. Soc.*, **94**, 3102 (1972); (b) M. C. Khosla, R. R. Smeby, and F. M. Bumpus, *J. Amer. Chem. Soc.*, **94**, 4721 (1972); (c) M. Rothe and J. Mazanek, *Angew. Chem., Int. Ed. Engl.*, **11**, 293 (1972).
(31) While this work was in progress, the syntheses of TRH and analogs on benzhydrylamine supports were reported: (a) J. M. Stewart, M. Knight, A. C. M. Paiva, and T. Paiva in "Progress in Peptide Research," S. Lande, Ed., Gordon and Breach, New York, N. Y., 1972, p 59. (b) J. Rivier, W. Vale, M. Monahan, W. Ling, and R. Burgus, *J. Med. Chem.*, **15**, 479 (1972).
(32) G. Flouret, *J. Med. Chem.*, **13**, 813 (1970).
(33) A. Anastasi, V. Erspamer, and J. M. Cei, *Arch. Biochem. Biophys.*, **108**, 341 (1964).
(34) While this work was in progress, a preliminary communication of the synthesis of substance **P** on the benzhydrylamine support was reported: G. W. Tregear, H. D. Niall, J. T. Potts, Jr., S. E. Leeman, and M. M. Chang, *Nature New Biol.*, **232**, 87 (1971).
(35) V. Erspamer, G. Erspamer, and M. Inselvini, *J. Pharm. Pharmacol.*, **22**, 875 (1970).
(36) T. Nakajima, T. Tanimura, and J. J. Pisano, *Fed. Proc.*, **29**, 282 (1970).
(37) G. L. Callewaert, R. Shipolini, and C. A. Vernon, *FEBS Lett.*, **1**, 111 (1968).
(38) E. Habermann and J. Jentsch, *Hoppe-Seyler's Z. Physiol. Chem.*, **348**, 37 (1967).
(39) (a) L. Yu. Skylarov and I. V. Shaskova, *Zh. Obshch. Khim.*, **39**, 2779 (1969); (b) J. Meienhofer and A. Trzeciak, *Proc. Nat. Acad. Sci. U. S.*, **68**, 1006 (1971); (c) J. D. Glass, I. L. Schwartz, and R. Walter, *J. Amer. Chem. Soc.*, **94**, 6209 (1972).
(40) A. I. Vogel, "Textbook of Practical Organic Chemistry," 3rd ed, Longmans, Green and Co., New York, N. Y., 1964.
(41) J. Scotchler, R. Lozier, and A. B. Robinson, *J. Org. Chem.*, **35**, 3151 (1970).
(42) C. Y. Bowers and A. V. Schally in "Hypophysiotropic Hormones of the Hypothalamus," J. Meites, Ed., Williams and Wilkins, Baltimore, Md., 1970.
(43) S. Shaltiel and M. Fridkin, *Biochemistry*, **9**, 5122 (1970).
(44) L. M. Pourchot and J. J. Johnson, *Org. Prep. Proced.*, **1**, 121 (1969).

Reactions Catalyzed by $Co_2(CO)_8$. Selective Deuterium Incorporation into Some Polycyclic Hydrocarbons

Thomas A. Weil

Department of Chemistry, University of Chicago, Chicago, Illinois 60637

Sidney Friedman* and Irving Wender

Pittsburgh Energy Research Center, U. S. Bureau of Mines, Pittsburgh, Pennsylvania 15213

Received March 15, 1973

Anthracene and anthraquinone can be converted to 9,9,10,10-tetradeuterio-9,10-dihydroanthracene in the presence of excess D_2 (or D_2O), CO, and catalytic amounts of $Co_2(CO)_8$. Pyrene also undergoes reduction and H-D exchange at the 4 and 5 positions; phenanthrene fails to react. When 1-octene is treated with CO and $Co_2(CO)_8$ in the presence of the tetradeuteriodihydroanthracene, as a deuterium source, C_9 aldehydes, randomly substituted with deuterium, result.

Both heterogeneous and homogeneous metal catalytic systems effect random hydrogen–deuterium exchange between water (or D_2O) and alkylbenzenes, benzene, and polycyclic aromatics.[1-7] Garnett and coworkers have extensively studied the homogeneous aqueous system for H-D exchange based on $PtCl_4^{2-}$.[2-7] Recently, a homogeneously catalyzed exchange between deuterium gas and an aromatic compound has been reported.[8]

We wish to report that $Co_2(CO)_8$ will catalyze selective hydrogen–deuterium addition and exchange involving anthracene (or 9,10-dihydroanthracene) and anthraquinone with either deuterium gas or D_2O. To our knowledge, this is the first example of an exchange catalyst that has high selectivity for specific hydrogens and that can use either D_2 or D_2O as its isotope source.

When anthracene is treated with synthesis gas and catalytic amounts of $Co_2(CO)_8$,[9] quantitative yields of 9,10-dihydroanthracene are obtained.[10] The reduction has now been done using D_2 or D_2O, CO, and $Co_2(CO)_8$. We find, accompanying the reduction of anthracene, exchange at the 9 and 10 positions takes place, resulting in formation of 9,9,10,10-tetradeuterio-9,10-dihydroanthracene (I). The same product is formed when anthraquinone is treated with D_2 or D_2O in the presence of CO and $Co_2(CO)_8$. This reaction, using successive equilibrations, offers a synthetic route to I. By chemical, rather than catalytic, dehydrogenation of I, it should be possible to prepare anthracene isotopically labeled on the 9 and 10 carbon atoms (V).

Experimental Section

Mass spectral analysis was done on a Consolidated Electrodynamics Corp. Model 110-B high-resolution instrument.[11] The inlet system was operated at 160°[12] and 10^{-6} Torr. It has been shown that deuteration does not change the sensitivity of the component in the mass spectrometer.[13] The data therefore are shown as a per cent of total ionization, which assumes equal sensitivities for all components.

Nmr spectra were run on a Varian A-60 spectrometer with chemical shifts reported in parts per million from TMS (δ).

Reduction–Exchange of Anthracene Using $Co_2(CO)_8$, and D_2. To a 200-ml rocking autoclave were added 0.2 g of $Co_2(CO)_8$, 2 g of anthracene, and 30 ml of benzene. The system was flushed several times with CO and pressured with a mixture of D_2 (98 atom %) and CO (see Table I). Upon completion of the reaction, the mixture was refluxed in air for 8 hr to decompose the $Co_2(CO)_8$ and filtered, and the solvent was removed. The product was recrystallized from ethanol.

Reduction–Exchange of Anthracene Using $Co_2(CO)_8$ and D_2O. To a 200-ml rocking autoclave were added 0.2 g of $Co_2(CO)_8$, 2 g of anthracene, 30 ml of dioxane, and 5 ml of D_2O. The system was flushed several times with CO and pressured to 3000 psi with CO. The work-up of the reaction product was the same as above.